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An Economic Appraisal of Microgravity Protein Crystallization for Drug Development

Troy J. Scott
RTI International
Research Triangle Park, NC

Nicholas S. Vonortas
Institute for International Science and Technology Policy
& Department of Economics
The George Washington University
Washington, DC

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Executive Summary

Understanding the structures of proteins can aid in understanding their function, including the role they play in disease. Structures can thus enable and accelerate the development of drugs that target those proteins. Although technological advances have greatly reduced the difficulty of determining protein structures over the past two decades, the production of diffraction-quality protein crystals remains a major obstacle to determining the structure of numerous medically interesting complex proteins, notably membrane proteins. Although protein crystallization experiments in the microgravity environment of low-earth orbit have produced crystals that diffract to higher resolution than crystals of the same proteins grown on earth, the research community has yet to reach consensus on whether these differences have made meaningful contributions to medicine, and whether the potential for future contributions justifies investment.

Interviews with scientists in the pharmaceutical industry, academia, and government point to crystallization of the most challenging medically interesting proteins as the most promising use case for microgravity in this subfield. In this application, microgravity will need to demonstrate its value in comparison to the output of structural biology centers that specialize in the most difficult crystallization problems, benchmark metrics for which are available from the National Institutes of Health Protein Structure Initiative. The Structural Genomics Consortium provides a model for partnering with industry on such efforts, elements of which could be adapted for microgravity protein crystallization efforts. Industry peer review, for example, could help especially in quantifying the incremental value of improvements in structure resolution, on which the value proposition of microgravity for drug development applications greatly depends.

We examine the case for microgravity protein crystallization under different private and public investment scenarios. The analysis suggests that sustaining investment is unlikely to come from individual companies. If microgravity is to make tangible contributions to drug development, public and private investment will need to be combined and managed to overcome a number of challenges. These include the

need to catch up to the indirect network externalities benefitting earth-based crystallography and the need to integrate microgravity crystallization into the complex system of technologies involved in structure-based drug design.

Protein crystallography has benefitted from several transformative technological advances since 2000. Although some innovations may offer at least the potential to complement microgravity (e.g., by enabling monitoring of crystal growth and *in situ* X-ray diffraction to be carried out on the International Space Station), most if not all realized improvements have had the effect of substituting for the contributions of microgravity. The development of microfluidics technologies has made it easier to grow small, high-quality crystals, and new-generation X-ray synchrotrons have made it possible to collect good data from extremely small crystals. Automation and high-throughput methods have increased the number of crystallization conditions that can be explored simultaneously in search of leads—conditions that can then be optimized to produce the best crystals.

These innovations have diminished the potential for microgravity to add value in two ways: first, by increasing the likelihood of obtaining high-resolution structures from crystals grown using the best-available earth-based technology, so that the likelihood of doing significantly better in microgravity is reduced; second, by generating such an abundance of structures of important proteins that the incremental value of 'the next' such structure is reduced.

The most promising potential applications of microgravity for drug development are most likely to be found in the earliest stages of drug development, where structural analysis can provide fundamental insight into the function of a protein or the mechanism of a disease, which can point the way to novel opportunities for drug design. In later stages, after a company has resourced a drug development project, set a timeline, and established formal milestones, the time needed to transport crystallization experiments to the International Space Station and return them to earth—even in a best-case scenario of three to six months—is too costly in most cases. Promising applications must also involve the most difficult and therefore most costly protein crystallization problems, where the expected cost of determining a structure on earth may run

into millions of dollars, making the cost of transporting experiments to the International Space Station potentially competitive.

We suggest an organizational approach and an analytical framework for identifying high-value applications for microgravity crystallization that might be good candidates for public and private co-investment. The approach involves industry peer review as part of a consortium of public and private stakeholders, based on the model of the Structural Genomics Consortium. The framework involves a comparison of stakeholder preferences over different attributes of protein structures—such as cost and informational content—with the technical tradeoffs among those attributes offered by the best earth-based and space-based crystallization technologies. Promising applications of microgravity are those for which the frontier of technical possibilities on the International Space Station reaches combinations of attributes that stakeholders prefer to the best set of attributes achievable on earth—or for which a portfolio of approaches that includes microgravity is preferred to one that does not.

Effective interdisciplinary collaboration will be needed to implement this approach and framework to determine what opportunities may remain for microgravity that can attract private investment. The sustainability of investment—and the viability of significant private investment—in microgravity protein crystallization for drug development applications will depend on the number of opportunities that can be identified and whether that number can be sustained at a high enough level as ground-based technologies continue to improve.

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1. Introduction

Knowledge of proteins' structures can enable and accelerate the development of drugs that work by targeting (i.e., binding with) those proteins and modifying their activity. To leverage these benefits, Pharmaceutical companies commonly integrate structural biologists and computational chemists into project teams during preclinical development.[1] Structure-based approaches are credited in the development of numerous drugs, including the transformative HIV protease inhibitors and the groundbreaking kinase inhibitor imatinib (Gleevec) for leukemia.[2]

Most protein structures are determined by X-ray crystallography, a process that requires protein crystals of suitable quality. The challenge of producing diffraction-quality protein crystals has spurred numerous innovations, including taking protein crystallization experiments into the microgravity environment of low-earth orbit, where reduced convection and other factors favor crystal growth.[3] Such experiments were conducted first aboard the space shuttle (Littke and John, 1984, and DeLucas et al., 1986) and later on the International Space Station (McPherson and DeLucas, 2015).

Microgravity has produced crystals that grow larger and diffract to higher resolution than crystals of the same proteins grown on earth.[4] Yet the research community has so far reached no consensus on whether these differences have made meaningful contributions to medicine, and whether the potential for future contributions justifies investment.

A review by the National Research Council (NRC, 2000) found the results from microgravity protein crystallization experiments to be inconclusive, characterizing improvements up to that point as more often

[1] For technical discussions, see Anderson (2003), Stevens (2003), and Jorgensen (2004).
[2] On the transformative nature of these two drug classes, see Kesselheim, Tan, and Avorn (2015). On the role of structure-based approaches in the development of these and other drugs see Kubinyi (1999), Redig (2001), Wlodawer (2002), Stevens (2003), Hardy and Malikayil (2003), Noble et al. (2004), and Vijayakrishnan (2009).
[3] On the sources of crystal improvement in microgravity and the history of microgravity protein crystallization, see Snell and Helliwell (2005) and McPherson and DeLucas (2015).
[4] In his historical review of protein crystallography, Giegé (2013, p. 6474) finds: "Overall, space grown crystals grow larger, and have more regular external morphology and better internal order with reduced mosaic spread, although contradictory results have been reported." Giegé cites the review by Judge, Snell, and van der Woerd (2005) of the first 20 years of microgravity protein crystallization, as well as Snell et al. (1995), Ng et al. (1997), Declercq et al. (1999), Carter et al. (1999), and Ng (2002). For contradictory results, he cites Sauter et al. (2012).

incremental than transformative. However, the NRC expressed optimism that new facilities then planned for the International Space Station would enable researchers to resolve the uncertainty around the value of microgravity, and it recommended that NASA focus its efforts there: "While past NASA-supported research on the crystallization process has not been without value, NASA's priority should now be to resolve questions about the usefulness of protein crystal growth in the microgravity environment to tackle important biological questions" (NRC, 2000, p. 18).

Interviews conducted in the context of the present study between July 2016 and March 2017 with scientists at pharmaceutical companies, in academia, and in government, revealed that a diversity of opinions endures; microgravity protein crystallization still has its optimists and its skeptics within each of these sectors today. Indeed, it seems that much of the NRC (2000) report remains true today. What has clearly changed since 2000 is that protein crystallography has been transformed by technological change. Advances in automation, microfluidics, and X-ray synchrotrons have greatly altered the landscape, reducing in many cases the potential for microgravity to add value.

This paper reviews these trends and the challenges they pose to identifying remaining cases where microgravity could be expected to add value. We also review the significant barriers to private investment in microgravity protein crystallization. The analysis is focused solely on the application of microgravity protein crystallization to problems of protein structure determination to aid in drug development; we do not attempt any assessment of the value of microgravity protein crystallization for scientific discovery generally.

The rest of the paper proceeds as follows. Section 2 draws on the predecessor of this paper to summarize the arguments for protein crystallization in microgravity as promoted by and debated within NASA circles. This Section concludes with speculation that an overly narrow focus on NASA's value proposition in past appraisals may have tended to obscure the significant changes in earth-based technologies available to the pharmaceutical industry. Section 3 focuses on these technologies and their rapid development during the past couple of decades or so. This Section concludes with a suggestion to identify high-value applications for microgravity crystallization—remaining avenues for microgravity to add value, which recent

transformative technological change has not yet foreclosed. Section 4 picks up this topic, focusing on the most promising potential applications of microgravity from an economic perspective: Where is microgravity most likely to be competitive with the best earth-based technologies in terms of its cost and the value it can provide to drug development efforts? Section 5 suggests an organizational approach and an analytical framework for identifying high-value applications for microgravity crystallization. The approach involves industry peer review, based on the model of the Structural Genomics Consortium. The framework involves a comparison of stakeholder preferences over different attributes of protein structures—such as cost and informational content—with the technical tradeoffs among those attributes offered by the best earth-based and space-based crystallization technologies. Section 6 concludes.

2. The Argument for Microgravity Protein Crystallization for Drug Development

A basic mission of NASA is to use the United States' segment of the International Space Station, designated a U.S. national laboratory, to facilitate the growth of a commercial marketplace in low Earth orbit for scientific research, technology development, observation and communications, and human and cargo transportation. Protein crystallization research is one of several potential commercial applications of the International Space Station in the biosciences that has long been promoted by NASA. CASIS, the manager of the U.S. national laboratory on the International Space Station, has argued that expanding research in protein crystallization could be a way to readily leverage an existing biosciences sector and develop it into a self-sustaining activity. Optimism in this regard is based on expectations that the Space Station's microgravity environment will prove important in the development of drugs to treat diseases such as arthritis, cardiovascular disease, multiple sclerosis, osteoporosis, cystic fibrosis, and cancer (Vonortas, 2015).

Numerous sources document the decades-long trend in declining pharmaceutical research and development (R&D) productivity.[5] Recent estimates suggest this trend has continued, with the average

[5] See, for example, Woodcock and Woosley (2008) and Scannell et al. (2012).

development cost per newly approved drug increasing by more than eight percent per year in real terms over the last decade (DiMasi et al., 2016). In 2004, the U.S. Food and Drug Administration (FDA) launched the Critical Path Initiative in part to address this declining productivity, specifically by pursuing scientific advances in the drug development process that would be commensurate with advances in basic science. At the root of the problem, according to FDA, the science and technology of early drug discovery steps had accelerated much more quickly than the means of translating discoveries from the laboratory into early clinical studies and completing clinical studies cost-effectively: "Despite extensive investment in basic biomedical science over the past three decades, there has been very little change in the science of the development process. The sophisticated scientific tools used in drug discovery and lead optimization are generally not utilized in the preclinical and clinical development stages. […] We are using the tools of the last century to evaluate this century's advances" (Woodcock and Woosley, 2008, pp.4-5). FDA's Critical Path Initiative called for a robust national infrastructure to "increase efficiency, predictability, and productivity in the development of new medical products" (U.S. FDA, 2006, i).

An argument could be made that a microgravity protein crystallization facility, as part of the U.S. national laboratory on the International Space Station, could be a valuable element of such an infrastructure: If the facility could produce higher-quality crystals, which would yield appreciably better structures of proteins of interest for drug development (i.e., more accurate structures, containing greater information relevant for drug development), and if this could lead to higher-quality preclinical drug candidates (i.e., having a higher probability of being found safe and effective in clinical trials), then such a facility could increase the efficiency, predictability, and productivity of drug development.

A crucial difficulty with this argument is that structure-based drug development mainly contributes to those very early drug discovery steps that have already advanced so rapidly, including the identification of promising molecules that bind to a target protein (hits), the refinement of those hits into lead candidates, and the optimization of those leads for further development. Once those leads move into preclinical development, structure-based approaches—and therefore microgravity's contributions to the quality of

those structures—are no longer relevant. In interviews conducted for this study, pharmaceutical industry experts in structure-based drug development explained that it would be inappropriate to attribute any improvement in transition probabilities, cycle times, or other aspects of preclinical or clinical development phases to the availability of higher-quality protein structures.

This is not to say that microgravity protein crystallization cannot contribute to improvements in the productivity of the drug development process, only that microgravity's potential value added is focused on development stages that have already benefitted from transformative technological changes. Among these: during the 1980s and 1990s, combinatorial chemistry increased by roughly 800-fold the number of drug-like molecules that could be synthesized per chemist per year and saw a commensurate expansion of compound libraries; since the mid-1990s, high-throughput screening has reduced by tenfold the cost of testing compound libraries against protein targets; since the first genome was sequenced in the 1970s, DNA sequencing has become more than a billion times faster (Scannell et al., 2012).

Computational, structure-based drug design is itself one such transformative innovation, and some advances in computational drug design have the potential to increase the value of high-quality structures. For example, more powerful software for virtually screening digital libraries of drug-like molecules against target proteins might yield more valuable insight if fed more accurate structural models of those target proteins. The development of such software would increase the demand for higher-quality structures and thus for means of producing them, including microgravity. However, the field of protein crystallography has itself been transformed by technological change over roughly the past couple decades. On balance, technological advances since the release of the National Research Council's review of protein crystallization on the International Space Station (NRC, 2000) have tended to foreclose potential opportunities for microgravity to add value. The next section discusses these issues in greater detail.

3. Recent Trends in Protein Crystallography: Raising the Bar for Microgravity

Protein crystallography has benefitted from numerous transformative technological advances since 2000. Although some innovations may offer at least the potential to complement microgravity—e.g., by enabling monitoring of crystal growth and *in situ* X-ray diffraction to be carried out on the International Space Station—most if not all realized improvements have had the effect of substituting for the contributions of microgravity. Among the most important innovations are automation and high-throughput methods; miniaturization (towards nanocrystallogenesis); microfluidic chips, 'PhaseChip,' and related technologies that allow *in situ* X-ray diffraction; sophisticated X-ray optics, ultrasensitive detectors and microbeams (Giegé, 2013; Moraes et al. 2014).

The development of microfluidics technologies has made it easier to grow small, high-quality crystals, and new-generation X-ray synchrotrons have made it possible to collect good data from extremely small crystals. Automation and high-throughput methods have increased the number of crystallization conditions that can be explored simultaneously in search of leads—conditions that can then be optimized to produce the best crystals: "Automation and miniaturization of the protein crystallization process have greatly contributed to the efficiency and effectiveness of the experimental technique. At present, integrated crystallization systems can perform more than 100,000 crystallization trials per day combined with robust automated visualization and powerful interface for data management" (Moraes et al., 2014, p. 81). Large-scale automation and associated information technology infrastructure have enabled laboratories to glean useful data from marginal crystals of multiple constructs of a target protein bound to many different ligands, an approach pioneered by the Structural Genomics Consortium (Gileadi et al., 2007).

The development of these technology platforms has driven the development of new approaches that utilize the new technology to best effect, improving efficiency and in turn driving the commercial development of hardware that is optimized for standard laboratory conditions. This virtuous cycle by which complementary product and process technologies have coevolved has tended to place microgravity at a

disadvantage relative to the best earth-based methods for determining a protein's structure.[6] An important determinant of whether microgravity can emerge favorably will be the degree to which state-of-the-art technologies can be adapted and optimized for use on the International Space Station.

A related issue is that the effect of innovations since 2000 has been to imbed the production of diffraction-quality crystals within an integrated technology system for the production and use of protein structures: "Besides automation of the crystallization trials and their monitoring, screening of recombinant protein expression, protein purification for crystallization, protein stability, image analysis, seeding and other optimization procedures, ligand soaking, crystal harvesting, and crystal mounting have also been automated [and] integrated systems have been installed near to synchrotron sources enabling *in situ* diffraction analyses" (Giegé, 2013, p. 6480). Such integrated systems may tend to lock out microgravity crystallization technology. The integrated system may overcome the inherent advantages of microgravity for crystal growth by, e.g., rapidly iterating through many crystallization conditions to improve crystals and *in situ* screening to select the best crystals for the most careful analysis.

Given the extent to which microgravity crystallography has been placed at a relative disadvantage by the coevolution of earth-based product and process crystallography technology and the integration of that technology into complex systems technologies specialized for drug discovery, the prospects for microgravity crystallization would benefit from an improved capability to predict when microgravity can provide a more favorable expected performance-to-cost ratio than the best earth-based methods—or at least by its inclusion improve the expected performance of a portfolio of methods. The value proposition of microgravity depends on being able to identify such cases, or at least justify the expectation that enough such cases can be identified in the future to justify the necessary investment in optimizing crystallography technology for microgravity.

[6] This 'virtuous cycle' effect is essentially an indirect network externality benefitting earth-based crystallography, analogous to a dominant computer operating system for which commercial software is developed.

4. Identifying High-Value Applications for Microgravity Protein Crystallization

The roles of computational, structure-based approaches to drug development are highly specific to certain early stages of the drug development process. The potential roles for microgravity turn out to be even more stage-specific. We therefore begin this section with an overview of the drug development process before turning attention to potential applications of microgravity protein crystallization.

4.1. Overview of the Drug Development Process

Most drugs work by inhibiting the activity of one or more proteins or cell structures. A protein on which a drug acts is called a drug target. The drug development process (Figure 1) begins with identification and validation of a potential target. At this initial stage, target validation might be better described as target confidence building—developing a body of evidence that the protein is instrumental in a disease and that modulating its activity is likely to lead to a promising therapeutic.

Figure 1. Drug Development Pipeline

Computational, structure-based drug design contributes principally to the three development stages following target identification and validation: hit identification (or target-to-hit), lead generation (or hit-to-lead), and lead optimization.

Hit identification involves screening libraries of compounds to identify those that bind to the target protein. Traditionally, this screening is performed using *in vitro* assays to chemically evaluate potential hits. Virtual screening uses software to compare digital models of the target protein's structure with digital compound libraries (i.e., databases containing digital structural models of drug-like molecules). A virtual screening step can be used to reduce the number of compounds that must be synthesized and screened *in vitro*, reducing cost (Stevens, 2003). Virtual screening requires digital structural models of the target

protein, and better structural models lead to higher confidence in the virtual screening results, allowing the subsequent *in vitro* screening steps to be that much more focused.

Lead generation and optimization is an iterative process that modifies hits to have greater affinity for the target and bind more selectively to it. The best of these leads are carried forward to be developed into preclinical drug candidates. Lead generation also involves *in vitro* testing, and again the simplest way to think about the value of having high-quality structures is that it reduces the amount of trial and error that needs to be done *in vitro*. For the iterative process of lead generation, it is useful to have complex structural models of the lead candidate bound with the target. Bound structures are evaluated, the lead is modified *in silico* (virtually) and then synthesized, the structure of the new lead bound with the target is obtained, and the process repeats. For this iterative process, it is important that crystallizing the bound structure not be a bottleneck. Therefore, flying such co-crystallization experiments (i.e., transporting them to and from the International Space Station) at this stage is not practical. However, flying unbound target proteins, and even flying targets bound to precursor molecules (crude drug-like molecules on which a lead might be based and later refined and optimized through the iterative process) before the iterative process is moving ahead on a strict timeline, can still provide useful insight.

Anderson (2003) discusses two other computational methods, in addition to virtual screening, by which structures can be used to generate hits. The first is inspection of naturally occurring compounds that bind to the target, designing modified versions of those compounds to act as inhibitors, and then synthesizing and testing those compounds. The second is de novo generation, wherein small fragments of molecules are positioned on the target, scored, and linked *in silico*. These constructed molecules are then synthesized and tested. These two methods illustrate the possibility of generating leads based on target structures that could not have been generated at all without structures of sufficient quality.

Optimized leads are taken forward into preclinical development, at which point structure-based approaches are left behind. In preclinical development, wide ranges of dosages are tested using *in vitro* (test tube and cell culture) and *in vivo* (animal) assays to develop preliminary efficacy and safety information. Subsequent clinical (human) testing comprises three phases. In Phase I, a small number of healthy

volunteers are tested to establish safe dosages and to gather information on absorption, distribution, metabolism, excretion, and toxicity. In Phase II, drug candidates are tested in human subjects who have the targeted disease or condition. Phase II trials involve larger numbers of subjects than Phase I (ranging from dozens to hundreds) and are designed to yield evidence of safety and preliminary evidence of efficacy. Phase III testing consists of one or more large-scale trials designed to establish evidence of efficacy and to uncover side effects that occur infrequently. Phase III trials involve the largest numbers of subjects, ranging from hundreds to thousands.

4.2. A Role for Microgravity?

Drawing on the preceding discussion, we distinguish between two broad use cases for protein structures in drug development. The first is to provide fundamental insight into the function of a protein or the mechanism of a disease, which can point the way to novel opportunities for drug design. The second is the iterative process of refining small molecules to become preclinical drug candidates (iterative structure-based lead optimization). The near-term potential contribution of microgravity is found almost exclusively within the first use case. In the second case, structures are typically produced in two to four weeks, which does not allow enough time to fly experiments to the International Space Station and return crystals to earth. At this stage of drug development, when a company has resourced a project, set a timeline, and established formal milestones, no amount of improvement—even the difference between having a very accurate structure versus none at all—can justify a six-month wait. If a structure cannot be determined in a matter of weeks, the project will proceed without the structure or terminate.[7]

The first use case admits more flexibility to tackle difficult structure-determination problems in longer timeframes, making it a better fit for microgravity. But private investment is drawn more to problems that are likely to yield to known methods, for which risk and timelines can be more easily managed by a single company.[8] The most challenging structure-determination problems have been the focus, over the past 15

[7] This message was emphasized repeatedly by the industry experts we interviewed.
[8] Pharmaceutical and biotechnology companies do of course accept high levels of technical risk in developing new drugs; that is essential to their business models. By contrast, solving the structure of a specific protein is never

years, of structural genomics centers, notably those of the Protein Structure Initiative, established in 2000 by the National Institutes of General Medicine Sciences within the National Institutes of Health (NIH) and ended in 2015, and the laboratories of the Structural Genomics Consortium, established in 2003 and ongoing. About 15 percent of funding for the Structural Genomics Consortium comes from its industry partners, the rest from government and charitable foundations like the Wellcome Trust (Ledford, 2010).

This funding allocation is indicative of the spillover gap—the amount by which the social return on investment exceeds the private—associated with tackling these difficult structure-determination problems. An immediate implication is that microgravity protein crystallization will need a mix of public and private investment: If the problem is difficult enough for microgravity to have a potentially significant effect, it is unlikely to be fully funded by private investment.

These two use cases are related in a way that unfortunately reduces the potential value of microgravity even for the first and most promising application. Iterative structure-based lead optimization relies on the ability to solve structures relatively quickly. This is done more easily when the protein has been crystallized successfully, and that successful experiment can be replicated. The target protein bound to a slightly modified small molecule can be expected to crystallize under conditions close to those found to work best for the same protein and a precursor version of the small molecule. Thus, a novel structure is only part of the output of a successful earth-based crystallography project; that output also includes a roadmap for the iterative process that a drug company will want to use in developing a drug for that target protein. Crystallization experiments in microgravity will be more difficult to replicate on earth. The very reason that microgravity can often produce better crystals—the environment being less demanding of crystallization conditions—is a disadvantage for producing the information needed to replicate a successful crystallization experiment on earth. Nor is replication only a concern for iterative structure-based lead optimization. Solving the structure of a protein bound to many different small molecules can help in understanding its function and is therefore a central part of fundamental scientific investigations.

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essential to a single company, and solving the most recalcitrant proteins' structures does not fit within the business models of most companies.

4.3. Introducing Cost Considerations

For this part of the discussion, we narrow our focus on the microgravity use case that emerged from expert interviews as having the most promising value proposition: a means of solving the most difficult medically interesting protein crystallization problems—before any specific drug development project is resourced and proceeding per a strict schedule. In this application, microgravity will need to demonstrate its value in comparison to the output of structural biology centers that specialize in the most difficult crystallization problems, benchmark metrics for which are available from the NIH Protein Structure Initiative and various other sources.

The average cost of determining a protein structure can range from less than $100,000 to $300,000, depending on the difficulty of the protein and the efficiency of the laboratories (Chandonia and Brenner, 2006). In its first six years, the Structural Genomics Consortium solved 1,000 novel structures at an average cost of $150,000 per structure (Ledford, 2010). These average costs conceal wide variation driven by the difficulty of the structure-determination problem. A decade ago, average costs of determining a novel structure were $140,000 for soluble bacterial targets, $450,000 for soluble human proteins, $1.5 million for bacterial membrane proteins, and $2.5 million for human membrane proteins (Stevens, 2003).

Costs range even higher for solving the structures of proteins that are least like those for which structures have already been solved. Proteins are made up of functional regions, or domains, that are repeated in different combinations to give proteins their unique properties. Once some or all of a protein's domains have been solved, the full structure becomes easier to solve. A decade ago, the average cost of solving the first-ever structure in a protein domain family ranged from $1.5 to $5.5 million; the average cost of solving the first-ever structure in a superfamily ranged from $2.0 to $7.3 million (Chandonia and Brenner, 2006). These costs have surely fallen with the technological changes of the last decade, but the important point is that costs remain much higher for the most difficult proteins, and these can often be identified in advance.

The high cost for the most difficult cases reflects the costs of many failures, in a trial-and-error process, prior to the first success. One way in which microgravity could add value is by reducing the number of

different conditions that must be tried before the first successful crystallization, thus reducing this cost. For the most difficult proteins, for which the expected cost of structure determination is highest, the cost of transporting the crystallization unit to and from the International Space Station might be comparable to the cost of the trial and error that could be avoided in a microgravity environment more forgiving of less-than-optimal crystallization conditions.

The cost of flying one International Space Station protein crystallization unit, which holds about 1,000 crystallization samples (25 lbs) plus the incubator containing the proteins (80 lbs) is about $1.5 million (Vonortas, 2015). It would still be important to consider for such cases that a successful microgravity experiment could not discover the conditions needed to replicate crystallization on earth, without which the structure may be less valuable to the research community and drug companies.

4.4. Valuing the Impact of Protein Structures

The preceding section argues that microgravity protein crystallization is more likely to be attractive when obtaining protein structures from earth-based crystallization experiments is expected to be most expensive. For private investment in the generation of such structures to be worthwhile, the structures themselves must be expected to add value to drug development efforts. In this section, we present some illustrative scenarios, using a tool to help in estimating the expected value of a protein structure, conditional on its effect on parameters of the drug development process: an expected net present value (ENPV) model of a drug development program.

We begin with a numerical example that conveys an important lesson: If direct cost savings in early stages of drug development are the only impacts of microgravity protein crystallization, then the business case will be difficult to make as long as the outcome is uncertain and delays are long. The reason is that, for projects with high ENPV, delay is too costly, while lower-valued projects are more likely to simply be abandoned in favor of higher-value ones.

The setting for this example is as follows: crystals are grown in microgravity that either could not have been grown on earth or that provide structural models of substantially higher quality than could have been gleaned from earth-growth crystals. Having these structures enables researchers to apply the methods of

structure-based drug design, which reduces the cost of hit identification and lead generation. Interviews with pharmaceutical industry experts indicated that having quality structures relevant for a given discovery project could reduce the cost of hit identification and lead generation by roughly $250,000 to $1 million, or by between 6% and 25%. Think of this as a company's maximum willingness to pay for the certainty of having, without delay, high-quality structures that it could not have otherwise obtained, and that are relevant to a discovery project it certainly means to pursue.

Consider the following scenario, from the perspective of the pharmaceutical company:

- We know that we cannot get a good enough structure in our own laboratory to make structure-based approaches feasible.
- We think there is a 25% probability—given that we failed to produce good enough structures in our own laboratories—that crystallization experiments performed in microgravity will produce good structures.
- We anticipate three to six months between making the decision to fly experiments and getting them back. This is based on one to two months prior to flight providing NASA notice of what we intend to fly, then two to four months for a cycle of launch and return.[9]

Because the probability of realizing the $250,000 to $1 million savings by flying the experiments is 25%, the expected savings is $62,500 to $250,000. Assuming a 10.5% cost of capital, the cost of a 3-month delay is roughly $25,000, and the cost of a 6-month delay is roughly $49,000, per $1 million expected net present value of the discovery project.[10] At the upper end of the range of expected savings, even a three-month delay offsets the expected savings for a project with an ENPV of at least $10 million.

[9] Based on correspondence with CASIS, the 2018 International Space Station (ISS) commercial resupply contract provides for a total of seven launches and five returns: five SpaceX launches, each of which carry cargo to the ISS and return cargo to earth, and two Orbital launches which only carry cargo to the ISS. NASA ISS National Laboratory office has spoken publicly about expecting at least one launch per month having return capability beginning in late 2019.

[10] To allow continuous discounting, a 10.5% annual discount rate is converted to a 9.98% continuously compounded annual discount rate (the natural log of 1.105 is 0.09985). Then, $1,000,000$e^{-0.09985(3/12)}$ = $975,348, so the cost of a 3-month delay is $24,652, and $1,000,000$e^{-0.09985(6/12)}$ = $951,303, so the cost of a 6-month delay is $48,697.

A more compelling business case may be available in a situation where the ENPV of a viable lead would be large, but there is no realistic path to a viable lead without structures of higher quality than can be obtained conventionally. In such cases, transporting crystallization experiments to the International Space Station could offer some probability of realizing a high net present value that is not otherwise available. The time required to fly and recover the experiments reduces this value, but the alternative to realizing the value with a delay is not to realize it at all, or to do so only with a lower probability.

The essential feature of this scenario is that microgravity crystallization can increase the ENPV of a discovery project by increasing the probability of success—leveraging a high expected return conditional on success—rather than only reducing cost directly. To model this scenario in a useful way, we will consider a stylized version of it:

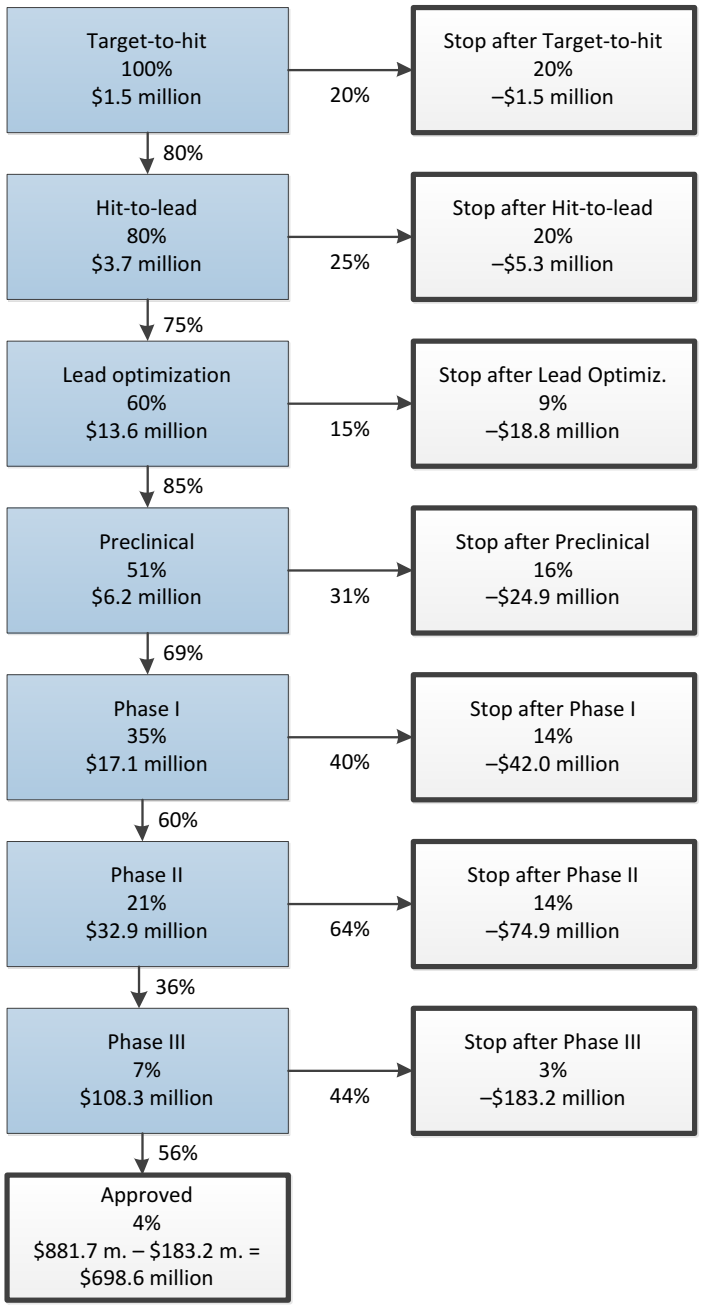
- Assumption 1: Baseline parameters (i.e., without microgravity crystallization) of the discovery process are as given by DiMasi et al. (2016), except in the details of preclinical development steps, not provided by DiMasi et al., for which we will use the parameters given by Paul et al. (2010).
- Assumption 2: Decisions about microgravity crystallization are made at the beginning of a discovery project, which we will call Time 0; for purposes of calculating ENPV, Time 0 is expected to be 128 months before the date of drug approval.
- Assumption 3: Baseline ENPV of the project is zero. This is equivalent to saying that the expected profits from a new drug are just equal to the average cost per new drug approved.

Assumption 3 is justified by recognizing that for a company to embark on the project it must expect the profits to at least cover the costs. We consider a situation in which the company is actually indifferent about the project without microgravity, and we model the impact of microgravity as increasing certain transition probabilities, thereby increasing the overall probability of realizing the payoff from a successful new drug approval. The resulting increase in ENPV will be discounted back to Time 0 to reflect the value of these downstream impacts at the time decisions about microgravity crystallization are made.

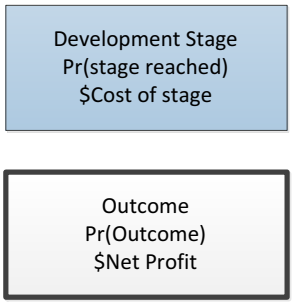
DiMasi et al. (2016) estimate the average cost per new drug approved to be $2,558 million, capitalized at a real rate of 10.5% per year to the date of approval. At Time 0, the expected present value of a successful drug approval is therefore $(\$2{,}558)e^{-r(128/12)} = \$882$, where $r = \ln(1.105) = 0.09985$ reflects an annual real discount rate of 10.5% with continuous compounding.

Figure 2 shows the baseline model with zero ENPV. The probability-weighted average of net profits in the eight outcome boxes (seven unsuccessful and one successful) is zero. Figure 3 summarizes the increase in ENPV that would result if the transition probabilities (positioned beside downward arrows in Figure 2) were to increase. Notice in Figure 3 that increasing transition probabilities by a given amount in later stages is more valuable. Intuitively, avoiding later failures is more valuable because they are more costly, coming after more investment costs have been sunk. The ranges of values considered in Figure 3 are hypothetical. Projects for which microgravity crystallization could be expected to have such impacts may be uncommon, and some of the numbers may not be plausible. Indeed, experts we spoke to emphasized that it would be inappropriate to attribute any improvement in transition probabilities of later development phases to the availability of higher-quality protein structures. These values are intended only to illustrate possibilities. All values would need to be discounted by the probability that microgravity crystallization would in fact produce satisfactory results. The cost of waiting for results would also need to be considered.

Figure 2. Baseline Drug Development ENPV Model



Key:



Notes:

This model is parameterized based on DiMasi et al. (2016), estimating the average cost per new drug approved to be $2,558 million, capitalized at a real rate of 10.5% per year to the date of approval.

All dollar amounts are discounted to Time 0, at the beginning of the discovery/development project, 128 months before the expected date of approval.

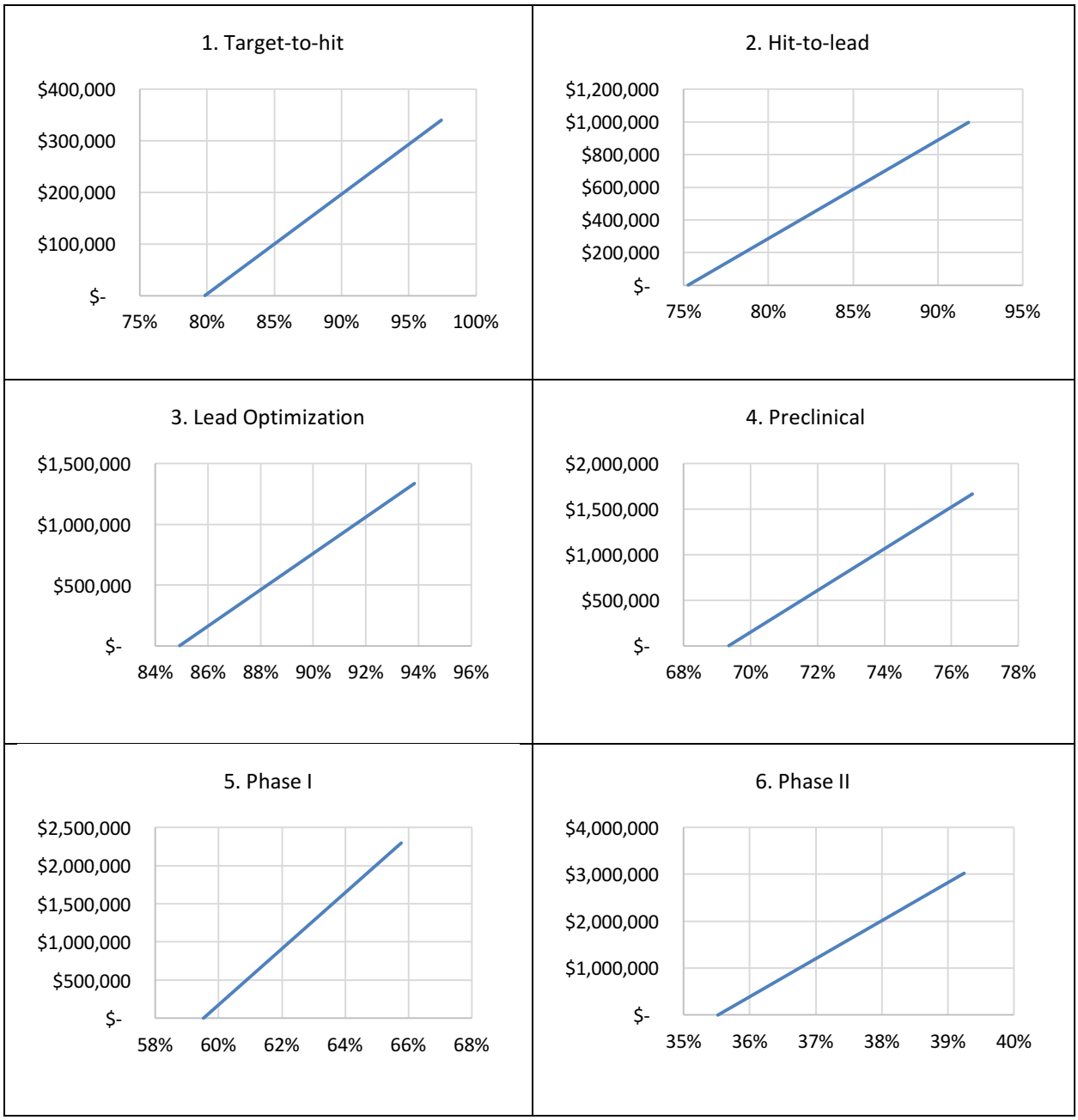
For the model, we assume that the expected value of approval is exactly equal to the expected cost: $2,558 million at the date of approval, or $881.7 million discounted back to Time 0.

The detail information in preclinical phases is based on Paul et al. (2010).

DiMasi, J. A., Grabowski, H. G., & Hansen, R. W. (2016). Innovation in the pharmaceutical industry: new estimates of R&D costs. Journal of health economics, 47, 20-33.

Paul, S. M., Mytelka, D. S., Dunwiddie, C. T., Persinger, C. C., Munos, B. H., Lindborg, S. R., & Schacht, A. L. (2010). How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nature reviews Drug discovery, 9(3), 203-214.

Figure 3. Sensitivity of ENPV to Improvements in Transition Probabilities



Note: The x-axis shows the probability of transitioning to the next phase. The y-axis shows the improvement in ENPV at Time 0 (start of the discovery/development project). Baseline probabilities correspond to the intersection of each sloping line with the x-axis, where ENPV = 0.

5. Assessing Additionality of Microgravity for Medical Innovation

Microgravity protein crystallization experiments have undoubtedly produced results that are different in many cases from earth-based controls, most notably in terms of the size, shape, and internal order of the crystals (Giegé, 2013; Judge et al. 2005). More difficult, however, is to quantify the impact of a given improvement achieved in microgravity on drug development.

Typically, structures' contributions to drug development are less visible than in the examples of kinase and HIV protease inhibitors, coming as they do in the earliest stages of drug development, often 10 years or more before a drug is approved, when as few as one in 20 early-stage drug discovery projects will ever reach approval stage. Attributing impacts to a specific set of microgravity experiments is especially difficult.

As an example, in 2009, a human protein involved in Duchenne's muscular dystrophy was crystallized on the International Space Station bound to a small molecule inhibitor, HQL-79. Earlier, the same target protein and small molecule had been crystallized on earth, yielding a structure at 1.45 Å resolution (Aritake, 2006). The 2009 flight improved the resolution to around 1.0 Å. As of February 2017, Taiho Pharmaceuticals is conducting a Phase IIa study of a different inhibitor, TAS-205, against the same target; results have not yet been reported (ClinicalTrials.gov Identifier NCT02752048, last accessed on August 1, 2017). Whether TAS-205 is an optimized version of HQL-79 (also developed by Taiho) is not clear, but it is certainly plausible. If it is, it would still be difficult to determine with any precision what the impact of the 2009 improvement in resolution has been on the development project.

More realistic is to compare the performance of microgravity with earth-based experiments. An important open question is: What is the appropriate earth-based control? The answer depends on the question we are trying to answer.

Consider as an example, experiments flown in 1997 on space shuttle flights STS-83 and STS-94 produced parvalbumin crystals that diffracted to a resolution of 0.91 angstrom (Å), while control

experiments on the ground produced no crystals suitable for diffraction (Declercq et al., 1999).[11] However, ground based experiments had previously attained a resolution of 1.50 Å for parvalbumins, and ground-based experiments had yielded crystals of similar proteins diffracting at 0.85 Å (Declercq et al., 1999). The value and significance of this research was not to dramatically improve upon the best-ever-achieved structure for parvalbumin. Rather, parvalbumin was used as a model protein to demonstrate the difference made by microgravity. Such experimentation in microgravity even played a role in the transformational technological change that followed in the roughly two decades since, because it "stimulated new research lines aiming to simulate microgravity conditions on earth and to develop alternative methods of crystallization" (Giegé, 2013, p. 6471).

A different control is needed to demonstrate the value of microgravity for drug development. There, even showing improvement over the 'best-ever-achieved' structure is not a stringent enough test. A more convincing demonstration would be a head-to-head challenge, between microgravity and the most efficient structural biology centers, to solve a number of novel structures. The relative efficiency of microgravity could then be assessed against the centers by a peer review panel. Including industry participants in the peer review process could help to assess pharmaceutical and biotechnology companies' preferences over different outcome metrics, such as cost, resolution (or information content) of the structure, time, and replicability. The Structural Genomics Consortium provides a model for partnering with industry on peer review, elements of which could be adapted for microgravity protein crystallization efforts (Edwards, 2016).

Figure 4 depicts a situation in which microgravity is preferred over the best earth-based alternative, given expected costs and conditional on the informational content of the structures produced. Although it is more expensive (in the hypothetical situation shown) to produce the same resolution as would be produced on earth (shown at the point E^*), the higher-resolution structure produced at higher cost in microgravity is preferred (point mG^*).

[11] Resolutions at all less than 1.0 Å are considered excellent; resolutions less than 2.5 Å are very good. For many drug-development applications the improvement from 3.0 Å to 2.5 Å is especially meaningful.

Figure 4. Illustrative Example in which Preferences and Technological Opportunity Favor Microgravity

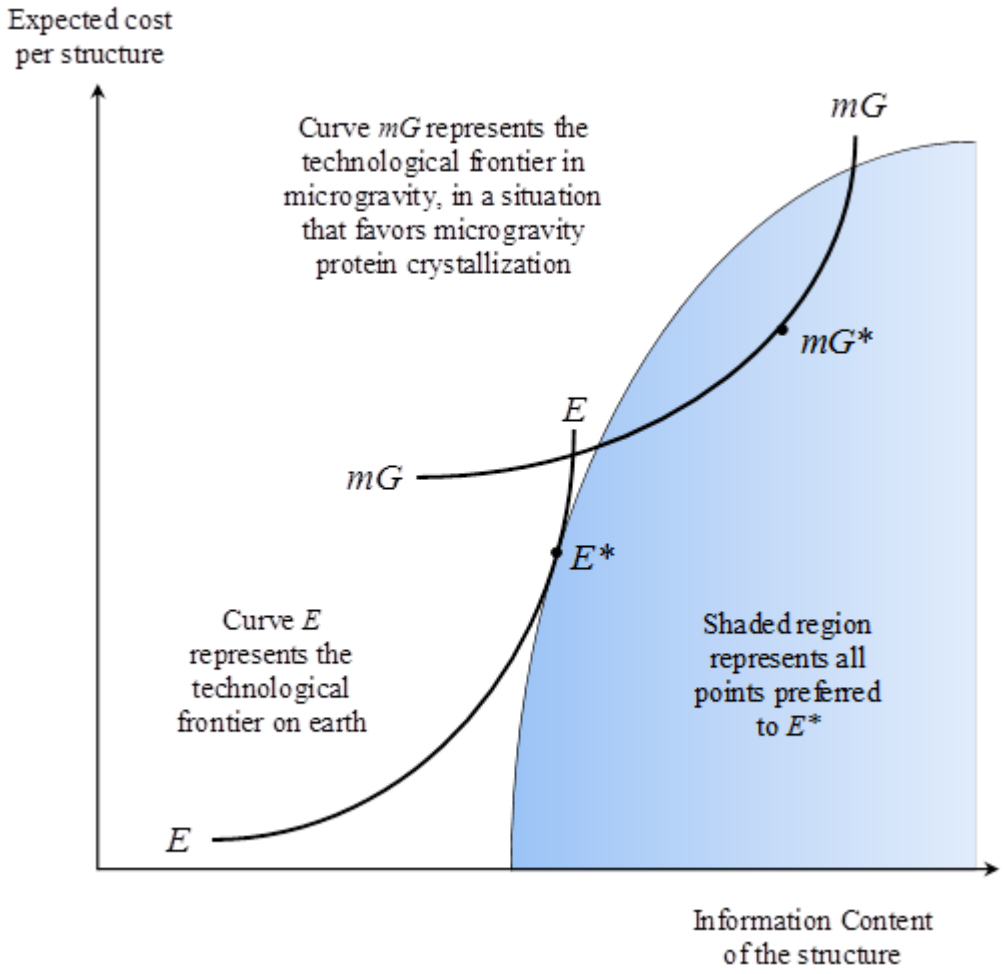


Figure 4 suggests a more general framework that may be useful in identifying the most promising applications of microgravity protein crystallization. The framework involves a comparison of stakeholder preferences over different attributes of protein structures with the technical tradeoffs among those attributes offered by the best earth-based and space-based crystallization technologies. The information content of protein structures and the expected cost per structure are two such attributes. The time required to obtain structures and the replicability of successful crystallization experiments are two others.

By pooling their expertise, a consortium of scientists from national laboratories, universities, and industry could map out preference relationships (like the boundary of the blue-shaded region in Figure 4)

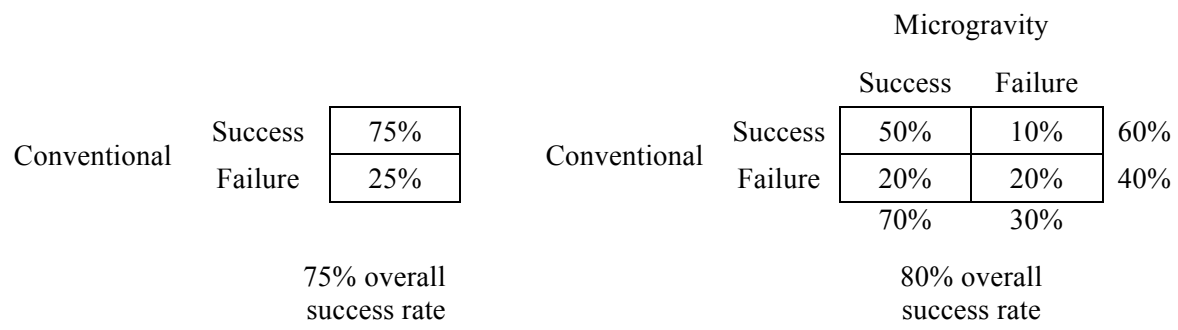
and technical tradeoffs (like the curves *E* and *mG*) in a higher-dimensional attribute space. Then, the search for promising applications of microgravity protein crystallization can be directed toward identifying cases like that depicted in Figure 4, where the frontier of technical possibilities on the International Space Station—or in microgravity generally—reaches points that some set of stakeholders prefers to their most-preferred point of any achievable on earth.

More subtly, and more realistically, microgravity could prove promising in applications for which a portfolio of methods that includes microgravity is preferred to one that does not. By its inclusion, microgravity could improve the performance of the portfolio, measured in terms of the amount of information about a protein's structure that can be generated in a given amount of time for a given cost, even if it is not expected to outperform every other approach in the portfolio.

The intuition can be seen with a simple numerical example, shown in Figure 5. In this hypothetical example, a portfolio of conventional, earth-based methods has a 75% success rate within a given timeframe and budget. If the same time and budget are allocated across conventional methods and microgravity, the portfolio has an 80% success rate. Even though, in the example, microgravity and conventional approaches each have a lower success rate in the combined portfolio than conventional methods did alone (60% for conventional approaches and 70% for microgravity), because the outcomes of microgravity and conventional methods are not perfectly correlated, the overall success rate of the portfolio is greater when microgravity is included.

This example suggests a refinement of the intuition conveyed by Figure 4, by simply recasting the curve *mG* to represent the technological frontier when earth-based methods and microgravity are combined. Figure 4 then depicts a situation in which the best portfolio that includes microgravity is preferred over the best earth-based portfolio. The essential insight is the same: If microgravity protein crystallization is to attract significant private investment, interdisciplinary teams of public and private stakeholders must be challenged to identify applications that can be realistically described in this way.

Figure 5. Numerical Example in which a Portfolio of Approaches that Includes Microgravity is Preferred



Note: In this hypothetical example, a portfolio of conventional, earth-based approaches has a 75% success rate within a given time and budget. If the same time and budget are allocated across conventional approaches and microgravity, the portfolio has an 80% success rate; the probability that either conventional approaches succeed, or microgravity succeeds, or both, is 10% + 20% + 50%. In the example, the success rate for conventional approaches falls to 60% when the same total resources are split between conventional and microgravity approaches. Yet, because the outcomes of microgravity and conventional approaches are not perfectly correlated, the overall success rate of the portfolio is greater when microgravity is included.

6. Discussion

As we have discussed in Section 3, protein crystallography has benefitted from several transformative technological advances since 2000—some in fact stimulated by early microgravity crystallography research. These innovations have made the search for promising microgravity crystallization applications more difficult in two ways: first, by increasing the likelihood of obtaining high-resolution structures from crystals grown using the best-available earth-based technology, so that the likelihood of doing significantly better in microgravity is reduced; second, by generating such an abundance of structures of important proteins that the incremental value of 'the next' such structure is reduced.

As an enhancement of crystallization capabilities for structure determination, microgravity crystallography projects must also compete with non-crystallization approaches to structure determination. Recently, cryo-electron microscopy has emerged as a viable and cost-effective technology platform, which could have significant impacts on the field of structural biology (Callaway, 2015).

Another headwind that proponents of microgravity protein crystallization should be aware of is a growing recognition of the possibility that, at least in some areas of medicine, the highly-directed search for drug-like molecules that bind with high affinity and specificity to a single target protein—the model of drug development that computational, structure-based approaches support—may in fact be less productive than the low-throughput *in vivo* screening and medicinal chemistry optimization approaches it has supplanted to a great extent since the 1990s (Scannell et al., 2012). The reason for this counterintuitive finding is that many drugs work by binding to multiple targets in complex biological systems, so that by focusing too narrowly on drug-like molecules with a high degree of specificity for a single target, and optimizing those molecules to further increase their specificity, companies may be overlooking more effective compounds and designing less effective drugs (Scannell et al., 2012, and Roth et al., 2004). The most promising applications for high-throughput and structure-based approaches—and therefore also for microgravity—may be in areas like oncology and others where diseases have relatively simple genetics, where therefore "molecular reductionism" may be genuinely helpful (Scannell et al., 2012).

One such example may be the search for better antidotes for nerve agents (chemical weapons and pesticides) that work by inactivating the enzyme acetylcholinesterase. Neutron crystallography, which requires especially large crystals that can be grown in microgravity, can yield insights into the reactivation of acetylcholinesterase that could enable the development of antidotes (Gerlits et al., 2016, and Kovalevsky et al., 2016).

To identify remaining cases where microgravity can be expected to provide better outcomes than the best earth-based methods, or where a portfolio of approaches that includes microgravity can be expected yield better outcomes than one that does not, stakeholders will need to understand trends in the evolving role of structure-based approaches to drug discovery, and how it may vary across therapeutic areas, as well as trends in technological change in protein crystallography and structural biology more generally, and the integration of crystallography into complex technology systems specialized for drug discovery. This technical understanding will need to be joined with understanding of the business models of pharmaceutical

and biotechnology companies, and the tradeoffs among time, cost, replicability, and information at different stages of drug development.

Effective interdisciplinary collaboration will be needed to bring these different pieces together and identify the best opportunities for microgravity protein crystallization that remain. This recommendation echoes Tassey (2015) in urging that NASA, with respect to its pursuit of the economic development of low earth orbit, "invest in a holistic technology development infrastructure that allows joint management with industry partners of R&D project portfolios" (Tassey, 2015, p. 20). In this context, we also strongly endorse the recommendation of Link and Maskin (2015) that NASA share the results of these collaborative efforts with prospective industry partners—and, we would urge, with the broader scientific community. The results to be documented and shared would include more than simply the protein structures gleaned from space-grown crystals and a comparison of these with earth-grown controls. Rather, to encourage private investment by reducing uncertainty around firms' expected rates of return, as Link and Maskin (2015) suggest, information shared would need to include description of the process of selecting proteins for flight, quantitative description of the expectations—ideally including quantification of associated uncertainties—supporting decisions to fly these proteins, comparison of outcomes with these expectations, and discussion of knowledge gained and recommendations for future experiments.

The Structural Genomics Consortium may provide a useful model for such collaboration, bringing together university and industry scientists, openly sharing results, and drawing funding from a mixture of private industry, private foundations, and government. A key element of the Structural Genomics Consortium model, which could be especially helpful in increasing the share of private investment supporting microgravity protein crystallization projects, is industry peer review. In the case of the Consortium, an independent advisory board of university and industry scientists sets quality criteria in advance and measures research outputs against them. "This external body prevents us from loosening the quality criteria for research outputs if achieving the original goals turns out to be harder than expected" (Edwards, 2016, p. 300).

That only about 15 percent of funding for the Structural Genomics Consortium comes from industry (Ledford, 2010) is indicative of the nature of the most difficult structure-determination problems, on which the Consortium focuses and which are also the most promising use cases for microgravity in this subfield, and should serve to set realistic bounds on expectations for the commercialization of microgravity protein crystallization for drug development. The sustainability of investment, including and especially the successful cultivation of private investment, will depend on the success of interdisciplinary collaborations in identifying opportunities—and establishing a realistic expectation of a continuing pipeline of opportunities—where microgravity can add value, even as ground-based technologies continue to improve. We hope that the analysis offered here can help steer such efforts in productive directions.

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